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Stem cells and genetic disease

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Abstract. Stem cell research is now a very broad field encompassing cells derived from all stages of life from the embryonic stem cells of the early blastocyst through to the adult stem cells of many tissues of the body. Adult stem cells from a variety of tissues are proving to be pluripotent and can differentiate into cell types different from the tissues from which they derive. Pre-clinical animal models indicate that adult stem cells do not cause tumours, not even, teratomas when transplanted. These properties, combined with the possibility of autologous transplantation, indicate significant advantages over embryonic stem cells in many proposed clinical applications.

Adult stem cells are already being used in treating genetic diseases. Bone marrow transplants and the haematopoietic stem cells within are used to treat genetic and acquired diseases of the blood and immune system. New developments include genetic engineering of haematopoietic stem cells to cure some genetic diseases. Other adult stem cells are being explored in pre-clinical animal models for transplantation therapy in a variety of genetic diseases, for which developing cellular models is a major potential application. Stem cells can be obtained from patients with known diagnosis and indicated genetic mutation. With these, and cells derived from them, genetic and biochemical pathways altered by the disease can be investigated and compared to cells from healthy controls.

Our own work indicates that there are multipotent stem cells in the adult olfactory mucosa, the organ of the sense of smell. This stem cell normally regenerates olfactory sensory neurones but has the ability to differentiate into a wide variety of cell types of all embryological lineages. These adult neural stem cells are accessible by simple biopsy and can be cultured indefinitely. Already they are showing promise as cellular models for investigating Parkinson's disease and schizophrenia. The promise of stem cell therapy derives from the hope that they can differentiate into any cell type desired (Bianco & Robey 2001). If a dopaminergic neurone is required, then a stem cell will be differentiated then transplanted into the Parkinson's brain. If a cardiomyocyte is required then a stem cell will be procured then transplanted into the ailing heart. Some workers even speculate that they might one day be used to manufacture tissues and even whole organs (Bianco & Robey 2001). At present the science is still 'art' and most of the hopes remain

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fantasy. Nevertheless, with more modest aims, there are many potential avenues for stem cell therapies and some are already used clinically.

STEM CELLS AND CELL TRANSPLANTATION THERAPIES

The sources of stem cells are varied. Commonly, they are classified into 'embryonic', 'germ line' and 'adult' stem cells. Embryonic stem cells are derived from the early blastocyst, from those cells already programmed to form the embryo. Germ line stem cells are derived from the cells that give rise to the egg and sperm. Adult stem cells may be more correctly termed 'tissue' stem cells, as they are found within otherwise differentiated tissues in adult and foetus. Adult stem cells are thought to provide the basis of tissue regeneration, replacement and repair. The best studied of these is the haematopoietic stem cell that regenerates the blood cells throughout adult life (Mikkola & Orkin 2006).

A stem cell is defined as an undifferentiated cell that divides asymmetrically, making a copy of itself plus a progenitor cell whose progeny ultimately differentiate into cells of specific tissues. This gives the stem cell the properties of self-renewal and multipotency. In this lineage, stem cells are often termed to divide 'slowly', meaning infrequently, giving rise to multipotent progenitors that may divide 'rapidly' meaning frequently and with short cell cycle, as transit amplifying cells that make up the bulk of cell division in a repairing or regenerating tissue. These multipotent progenitors eventually give rise to cell-specific precursors that differentiate into a mature cell type after their last division. These precursors are said to be unipotent, being committed to differentiate into a predefined cell type. Stem cells thought to be able to give rise to all cells of the embryo are pluripotent. If they can additionally differentiate into the extraembryonic membranes and trophoblast, they are called totipotent. Embryonic stem cells are regarded to be totipotent. Adult stem cells may not be totipotent; this remains to be proven, but many show remarkable capacity to differentiate into multiple cell types not associated with the tissue from which they derive. Adult stem cells are often considered less useful than embryonic stem cells, because they have not been shown to be totipotent, merely multipotent. The usual test of this multipotency is to establish whether adult stem cells can transdifferentiate across lineage boundaries. For example, an adult stem derived from ectodermal tissue such as skin or nervous system, which differentiated into a mesodermal tissue such as bone or fat, is said to transdifferentiate in this way. Many adult stem cells are known to be multipotent; thus, having the capacity to give rise to cells of their normal tissue and also to cells from many other tissues not associated with their embryonic origin (i.e. ectoderm, mesoderm and endoderm). Thus, adult stem cells need not be totipotent to be useful for stem cell-based therapies (Erlandsson & Morshead 2006).

The utility of stem cells in transplantation therapies varies depending on several factors, only one of which is theoretical developmental potency. Foremost is whether the transplanted cell can achieve the purpose for which it is being proposed. This may not require even multipotency if a unipotent cell would be effective; skin repair is an obvious example. The second consideration is whether the transplanted cell will be rejected by the immune system. Being foreign tissue, embryonic stem cells are likely to be rejected if immune suppressive drugs are not prescribed to a patient (these are contraindicated as they are associated with morbidity). Immune rejection is unlikely when adult stem cells are derived from the same patient – an autologous procedure. When allografts are necessary, adult stem cells may offer a wider range of people from which to find a closely matched immune phenotype. Therapeutic cloning, the process of deriving embryonic stem cells whose nuclear DNA is donated by the patient requiring the stem cells, may

not solve the immune rejection problem either, as such cells will carry the mitochondrial DNA from the egg donor that is unlikely to be the mother of the patient. The third consideration in stem cell transplantation is the danger of uncontrolled growth of the transplanted stem cells. For example, embryonic stem cells can form teratomas when transplanted into animal models of disease. One approach is to differentiate the stem cells into a cell of interest before transplantation, but this is not always successful. Adult stem cells do not have this property of uncontrolled proliferation after transplantation, which may prove to be one of their most advantageous properties.

The discussion above suggests that, *a priori*, adult stem cells hold the greater promise for cell transplantation therapies, compared to embryonic stem cells: they are accessible in most patients, autologously transplanted, and are unlikely to initiate tumours or teratomas. Indeed, adult stem cells are currently being used or trialled for many human disease treatments, including human genetic diseases (Pessina & Gribaldo 2006).

STEM CELLS AND CELLULAR MODELS OF DISEASE

Development of cellular models of disease remains a major goal of stem cell research (Findikli *et al*. 2006). The underlying hypothesis is that the developmental potency of stem cells provides an ability to derive cells of interest for a particular disease that are not normally available for study. Analogy can be found in the cellular models of cancer provided by cancer cell lines. For most human diseases, there is no cellular model, although animal models exist. Cellular models are of particular interest for genetic diseases in which it is assumed the consequences of a gene mutation or deletion will manifest themselves in a cell-specific manner. Stem cells can potentially be initiated to provide cells specific for a disease of interest. For example, it might be possible to derive gabaergic neurones from stem cells derived from persons with Huntington's disease or dopaminergic neurones from persons with Parkinson's disease. These neurones could then be subject to analysis *in vitro* and provide information concerning the disease process in question, in the same way that cells derived from breast or lung cancers have led to understanding of the aetiologies of, and treatments for their related diseases. The phenotype of different individuals with genetic diseases varies, even monogenic diseases, indicating that other mutations relevant in specific tissues may influence the course of a disease (Summers 1996; Sidransky 2006). It would be a significant advantage, therefore, to develop cellular models from multiple individuals with different genetic backgrounds and examine the outcomes in different stem cell-derived tissues.

Developing cellular models for disease remains a significant motivation to develop therapeutic cloning, the production of embryonic stem cells after somatic cell nuclear transfer (SCNT), the cloning technique via which animals are cloned (Wakayama *et al*. 2001). This route to cellular models has numerous disadvantages, the foremost being that it is currently not possible and unethical for humans. Additionally, even in animals in which therapeutic cloning is an established practice, the derivation of embryonic stem cells is a long and inexact process, depending on random (stochastic) and chance factors (Wakayama & Yanagimachi 2001). Similarly, derivation of a new human embryonic stem cell line is a significant achievement, uncommon enough that some cell lines are given names like 'Envy' (Costa *et al*. 2005). These technical barriers are such that even when human therapeutic cloning is achieved, it is unlikely that many cell lines will be generated for any disease of interest. It is likely that the only therapeutically cloned cell lines will be those from patients with a known single gene mutation or deletion, because only these known diseases will be worth the investment of time to investigate them. Derivation of

therapeutically cloned cells from persons with diseases of unknown genetic aetiologies would be a riskier research investment.

Adult stem cells can be obtained relatively easily and require weeks, rather than months, to generate. Potentially they could be generated from multiple individuals with a certain disease, including those with known mutations and those without. Illnesses with similar clinical presentation may arise from multiple causes. For example, less than 5% of all cases of Parkinson's disease arise from known genetic mutations, mostly it arises as a familial form (Hardy *et al*. 2006), with no known genetic mutations. It is probable that common mutations will be identified, but it is also probable that many people develop Parkinson's via exposure to unknown environmental factors (Benmoyal-Segal & Soreq 2006). The puzzle with Parkinson's disease is to understand the cellular pathways that can explain genetic and environmental contributions to it; this will not be solved by studying stem cells and their progeny from persons with only single mutation form. It is more likely to be solved by identifying shared characteristics between cells from persons with and without known gene mutations, and comparing their responses to environmental factors identified from the epidemiology of the disease. Such a study is possible to perform *in vitro* with adult stem cells, as they are relatively easy to obtain and grow.

There are many sources of adult stem cells. They are suspected to be in all adult tissues that can self-repair. If all adult stem cells prove to be multipotent, the exciting possibility is that there is an appropriate adult stem cell available for repair of any tissue type, with the potential for investigation of genetic diseases and their treatment. For example, bone marrow stem cells may be best for blood and bone diseases, cartilage stem cells for joints and neural stem cells for the nervous system, each providing specialized tools appropriate for the task at hand.

GENETIC DISEASES

Genetic diseases are those in which deleted or mutated genes play a role in their cause, bearing in mind that both genes and the operating environment contribute to most diseases. In some cases a single gene mutation or deletion leads to a single disease. These human monogenic diseases currently number about 6000 and represent about 1 in 200 births. Inheritance of them may be dominant (in which carrying one copy of the mutated gene leads to obligatory disease) or recessive (in which both copies of a gene must be mutated for disease to be manifest). Some may be X-linked, carried on the X chromosome, so that in males the disease is dominant and in females recessive. Examples of monogenic diseases are cystic fibrosis, sickle cell anaemia, Marfan syndrome, Huntington's disease and hereditary haemochromatosis.

Many more diseases and disorders can be linked to genetic changes, as demonstrated by higher risks associated with closer genetic affiliation, but these are considered to be polygenic, with multiple genes of small effect interacting to lead to a disease phenotype, and even monogenic diseases that are influenced by expression of further other genes (Sidransky 2006). In these cases, genetic epidemiology indicates increased risks of disease linked to multiple regions. For example, breast cancer susceptibility genes lie on multiple chromosomes, with known associated genes only accounting for 5–10% of cases (Goldberg & Borgen 2006).

Other conditions are considered to be more environmental in initiation, in that exposure to pathogens, toxins or diet may be causative factors. In reality, there may be no clear boundary between the more genetic and the more environmental causes of disease, as the environment interacts with the phenotype leading to the deficiency, such that the same pathogen has a different effect on individuals with dissimilar genetic background (Brinton & Nathanson 1981). Thus, in

many diseases it is believed that genetic susceptibility will be combined with a certain set of environmental risk factors to manifest the complaint in a certain individual. Environmental risk factors may also include social and psychological factors, which are not obviously amenable to investigations *in vitro*.

Some genetic diseases are due to abnormalities in chromosome structure such as missing or extra copies, or translocations. Examples include Down's syndrome (trisomy 21), in which there are three copies of chromosome 21. Finally, some genetic diseases arise from mutations in the 13 genes of the mitochondrial genome, the non-chromosomal DNA of mitochondria.

HOW MIGHT STEM CELLS BE USEFUL TO CURE GENETIC DISEASES?

Intravenous infusions of whole bone marrow are used to treat patients with acute leukaemia, bone marrow aplasia and congenital immune deficiencies (Ballas *et al*. 2002). The success of these treatments depends on properties of the haematopoietic stem cell, a rare cell that provides the model for all adult stem cell research. With the development of methods to isolate and characterize adult stem cells from various tissues though, new cell-based therapies to counter a range of genetic diseases are being developed, not only for those of the blood (Bordignon 2006). The most intuitive use of stem cells in genetic disease is for cellular repair of tissues affected by the genetic mutation, and stem cells without the mutation would be transplanted to restore normal function having been derived from a non-mutated, but otherwise tissue-matched donor. Alternatively, the stem cells might be derived from the patient, but be genetically engineered before re-implantation (Fischer *et al*. 2004). Stem cells have the potential to differentiate into the desired cell type to restore normal tissue function after transplantation; they may also be useful as cellular models to investigate genetic and biochemical pathways in cells when derived from persons affected.

In theory, therapy for monogenic diseases would involve replacing the faulty gene via genetic engineering. This simple concept is complicated, of course, that the effect of the mutated gene may have different cell and tissue consequences, depending on its level of expression. For example, the gene mutation responsible for cystic fibrosis is expressed in many epithelia and leads to a plethora of symptoms (Summers 1996), but gene therapy for it has targeted the epithelium of the lung, because it is accessible and because mucus overproduction is the leading cause of death. In contrast, it is hard to imagine a simple target for gene therapy for Huntington's disease, a degenerative condition that affects many brain regions, unless the therapy was applied to the germ line or to the early embryo. On the other hand, haemophilia and sickle cell anaemia are diseases of the blood. The genes responsible for these have restricted roles and therapy can be achieved by manipulating bone marrow stem cells responsible for production of the affected blood cell types (Sadelain 2006). It is perhaps not surprising, therefore, that the only stem cell therapies, including genetically modified stem cells, currently in clinical practice are those involving blood diseases (Bordignon 2006); all current therapies use adult stem cells, many being autologous in nature.

Stem cell therapy using genetically engineered cells will be most easily applied in monogenic diseases as only the single gene needs to be inserted or modified. Despite this, the therapeutic solution may not be obvious when the gene has actions in multiple tissues, requiring multiple sites of transplantation. For stem cell transplantation, generally, the most likely therapies will

arise where a single cell type can be transplanted into a single location. Some stem cell therapies might be curative, such as treatment of severe combined immune deficiency with genetically engineered haematopoietic stem cells (Cavazzana-Calvo *et al*. 2000), whereas others may only treat particular symptoms. An example of the latter would be transplantation of genetically modified gabergic neurones, derived from stem cells, into the brain striatum to treat the motor symptoms of Huntington's disease.

Adult stem cell biology has the potential to contribute to the understanding of genetic diseases and to assist in understanding gene–environment interactions by providing cellular models of disease in different individuals. Therapeutic cloning to provide embryonic stem cells has limited usefulness due to the technical difficulties in producing them and that they would be useful only in monogenic diseases. In contrast, adult stem cells are accessible and can be obtained from patients with known diagnoses, with a variety of genetic contributions. Adult stem cell-based cellular models will be used to identify genes and biochemical pathways involved in cellular pathology of genetic disease. This will be useful for developing biomarkers and diagnostic tests and for identifying new targets for drug treatments.

ADULT STEM CELLS ARE FOUND IN THE OLFACTORY SYSTEM

Research at our own laboratory concerns adult stem cells found in the olfactory mucosa, the organ of the sense of smell. This small patch of nervous tissue in the nose is easily accessible by biopsy through the external naris and contains stem cells and neural progenitor cells that continually regenerate and replace the olfactory sensory neurones, whose normal location makes them vulnerable to trauma from inhaled factors such as pathogens or chemicals (Mackay-Sim & Kittel 1991; Mackay-Sim & Chuah 2000; Mackay-Sim 2003). The rate of neurogenesis in the olfactory epithelium is very high, can be observed in humans at all ages (Murrell *et al*. 1996; Feron *et al*. 1998), and the stem cells are multipotent (Murrell *et al*. 1996, 2005; Feron *et al*. 1998). In the olfactory epithelium they reconstitute both neural and non-neural elements (Chen *et al*. 2004) and *in vitro* they can be induced to differentiate into numerous cell types, including neurones, glia, liver, kidney, heart, skeletal muscle and adipocytes (Murrell *et al*. 2005). In culture, they grow in characteristic neurospheres, which are tight clusters of cells containing stem cells, neural precursors, differentiating neurones and glia (Murrell *et al*. 2005) (Fig. 1a). The stem cells comprise less than 1% of these neurosphere cells, but they can be maintained *in vitro* for many generations. They are self-renewing and produce many millions of progeny. Olfactory neurosphere cultures can be stored frozen and can be recovered without apparent loss of multipotency.

One aim of our laboratory is to develop stem cell therapies using autologous stem cells from a patient's own nose (Fig. 2). We are currently undertaking a phase I clinical trial of autologous olfactory ensheathing cell transplantation in human spinal cord injury (Feron *et al*. 2005). These cells are not stem cells but are a specialized glial cells with similarities to both astrocytes and to Schwann cells (Mackay-Sim 2005). Pre-clinical animal models demonstrate their effectiveness in assisting repair after spinal cord injury (Mackay-Sim 2005). In the nose, the olfactory ensheathing cells assist regeneration and re-growth of the olfactory sensory neurone axons as they grow from the nose to the brain. Our on-going clinical trial indicates that transplantation of autologous olfactory ensheathing cells into the human spinal cord is safe at 1 year (Feron *et al*. 2005). We are now working on pre-clinical animal models of Parkinson's disease to determine the efficacy of olfactory stem cells in providing a source of dopamine after transplantation into

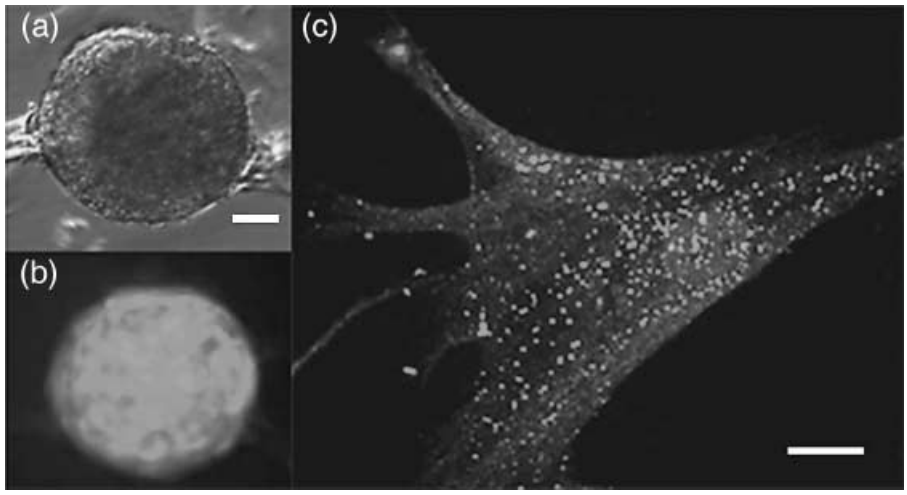

Figure 1. Adult stem cells from olfactory mucosa grow as neurospheres. (a) Neurosphere generated from human olfactory mucosa. Nomarski optics. Scale bar = 50 μm. (b) Fluorescence image of the same cells, genetically labelled with green fluorescent protein (GFP), under blue illumination. (c) Tyrosine hydroxylase-positive cell (green fluorescence) derived from a human olfactory stem cell. The nucleus is stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). Scale bar = 5 μm.

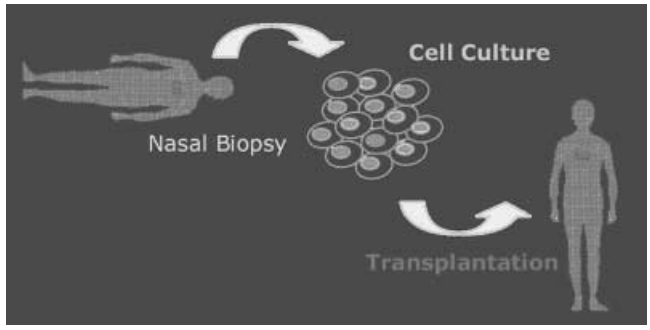

Figure 2. Cell therapies from olfactory mucosa. Nasal biopsies yield stem cells and olfactory ensheathing cells, a specialized glial cell with properties similar to Schwann cells and to astrocytes. These could be grown *in vitro* and transplanted autologously. Olfactory mucosa is accessible in adults and children.

the striatum. We are also exploring the potential of olfactory stem cells for transplantation after genetic manipulation. They can be transfected *ex vivo* using vector carrying foreign genes such as that for green fluorescent protein (GFP) and they retain gene expression for many months after transplantation into the brain (Fig. 1b). These experiments suggest that olfactory stem cells will be a useful source of cells for genetic manipulation and transplantation in therapies for genetic diseases.

Olfactory stem cells have the potential to provide cellular models of disease, because they are accessible via nasal biopsy in patients of all ages. In our laboratory, we now have olfactory neurosphere cultures from more than 50 patients including some with Parkinson's disease, motor neurone disease or schizophrenia. These cell lines are being developed as cellular models. For example, we are developing methods for differentiation into dopaminergic neurones for Parkinson's disease and into motor neurones for motor neurone disease (Fig. 1c). In schizophrenia, a disorder

of brain development, interest is in the fundamental aspects of cell proliferation and differentiation of stem cells. We have already shown that olfactory neurogenesis is altered in nasal biopsies in schizophrenia compared to control, with increased cell proliferation and altered gene expression (Feron *et al*. 1999; McCurdy *et al*. 2006).

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